



Decades-Old Theory of Enzyme Selectivity Challenged

"Three Point Landing" May Not Be Sufficient to Distinguish Optical Isomers

In a paper published in *Nature*, a Berkeley Lab group under the direction of Daniel E. Koshland Jr. in the CAM Biomolecular Materials Program used x-ray crystallography data to provide evidence that the long-accepted understanding of the specificity of an enzyme for one, but not the other, of a mirror image pair of molecules, may not be complete.

One of the hallmarks of the use of enzymes in materials synthesis or pollutant degradation is the exquisite specificity shown by these protein catalysts. In general, they bind to only one starting material (substrate) and convert it to only one product. This is an advantage in materials synthesis in that the starting pool of materials need not be pure, and no, or very limited separation of products and disposal of waste is required. In pollutant degradation on the other hand, this presents a challenge. Only a very limited array of structurally similar pollutant molecules will be destroyed by a particular enzyme. Enzymes would thus have to be designed to degrade other, less similar molecules.

One of the most impressive illustrations of this selectivity lies in the ability of the enzyme to distinguish between optical isomers of a given molecule (structures which have the same numbers, types and arrangements of atoms, but are simply mirror images of one another). The classical explanation of this phenomenon rests on the view that the enzyme presents a surface for the substrate (I) to bind so that it can be converted to product. Three of the four groups of atoms (A, B, and C in figure) attached to a central "tetrahedral" carbon atom (black in figure) of the substrate then make contact with that surface at three specific binding positions. This exposes the fourth group of atoms (D, in figure) for reaction. There is no way to rotate a mirror image (II) of that molecule to allow these same three groups to bind the surface and present the same fourth group for conversion to product.

Using x-ray crystallography data of the enzyme isocitrate dehydrogenase, an oxidation/reduction enzyme, the group showed that, as expected, under normal binding conditions, only the L-isomer of the substrate, isocitric acid, appeared bound to the enzyme, suggesting that the D-isomer bound weakly, if at all. On the other hand, and quite unexpectedly, when the binding was studied in the presence of Mg^{++} ions, only the D-isomer was seen bound in the active, or catalytic site.

Examination of the two structures revealed that the same three groups on the tetrahedral carbon of isocitrate bind the same sites on the enzyme (hatched areas in figure) in both situations. Because the two molecules are optical isomers, the fourth group, (D) points in opposite directions in the two.

Thus, three binding sites, A', B', C', are not sufficient to uniquely determine the mode of binding. Instead, true selectivity for one of a pair of optical isomers may sometimes require four constraints—the three binding sites and a fourth site that need be either a binding site or a directional constraint, for example restricting the direction from which the substrate can approach the surface of the enzyme. This result will be of great importance in attempts to design enzymes either for maximum specificity and restriction of substrates, or maximum flexibility in binding both isomers. In addition to its implications for materials synthesis and pollutants, this work could become a valuable tool in drug design where one isomer of a compound is therapeutic while the other is at best inactive, and at worst, toxic.

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Mesecar, A.D., D.E. Koshland, Jr., "A New Model for Protein Stereospecificity," *Nature*, **403**, 10 February 2000.